

Influence of NaCl Content and Cooling Rate on Outgrowth of *Clostridium perfringens* Spores in Cooked Ham and Beef^{†‡}

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MS 02-469: Received 19 December 2002/Accepted 23 March 2003

ABSTRACT

The effect of NaCl concentration and cooling rate on the ability of *Clostridium perfringens* to grow from spore inocula was studied with the use of a process that simulates the industrial cooking and cooling of smoked boneless ham and beef roasts. NaCl was added to ground cooked hams A and B (which were commercially obtained) to obtain levels of 2.4, 3.1, 3.6, and 4.1% (wt/wt) and 2.8, 3.3, 3.8, and 4.3% (wt/wt), respectively, and to raw ground beef to obtain levels of 0, 1, 2, 3, and 4% (wt/wt). Ham C, a specially formulated, commercially prepared product, was supplemented with NaCl to obtain levels of 2.0, 2.5, 3.0, and 3.5%. The samples were inoculated with a three-strain mixture of *C. perfringens* spores to obtain concentrations of ca. 3 log₁₀ CFU/g. Portions of meat (5 g each) were spread into thin layers (1 to 2 mm) in plastic bags, vacuum packaged, and stored at -40°C. Thawed samples were heated at 75°C for 20 min and subsequently cooled in a programmed water bath from 54.4 to ≤8.5°C in 15, 18, or 21 h. For the enumeration of *C. perfringens*, samples were plated on tryptose-sulfite-cycloserine agar and incubated in an anaerobic chamber at 37°C for 48 h. Population densities for cooked ham and beef increased as cooling time increased, and NaCl exerted a strong inhibitory effect on the germination and outgrowth of *C. perfringens*. For beef, while 3% NaCl completely arrested growth, pathogen numbers increased by ≥3, 5, and 5 log₁₀ CFU/g in 15, 18, and 21 h, respectively, when the NaCl level was <2%. *C. perfringens* did not grow during cooling for 15, 18, or 21 h in ham samples containing ≥3.1% NaCl. Results obtained in this study suggest that a 15-h cooling time for cooked ham, which is normally formulated to contain >2% NaCl, would yield an acceptable product (with an increase of <1 log₁₀ CFU/g in the *C. perfringens* count); however, for beef containing <2% NaCl, *C. perfringens* populations may reach levels high enough to cause illness.

Clostridium perfringens is an anaerobic, gram-positive, spore-forming bacillus. This organism is ubiquitous in the environment and is found in the intestinal tracts of animals and humans (7). Thus, raw protein foods of animal origin are frequently contaminated with *C. perfringens*. In one study, *C. perfringens* was found in 50% of ground beef samples and on 29, 66, and 85% of beef, pork, and lamb carcasses (13). The incidence of gastrointestinal illness due to *C. perfringens* infection in the United States has been estimated by the Centers for Disease Control and Prevention to be 248,000 cases per year, with 100% of these cases being due to foodborne transmission of the pathogen (15). The generally accepted temperature range for the growth of *C. perfringens* is 12 to 50°C (7). A unique characteristic of *C. perfringens* is its relatively high optimum growth temperature. At 43 to 47°C, the generation time for the organism is <10 min in meat (7). Willardsen et al. (26), in a study of growth and survival of *C. perfringens* vegetative cells in autoclaved ground beef at constantly rising tem-

peratures, found that the inactivation of the cells began at ca. 55°C and that the rate of inactivation increased as the temperature rose above 55°C. However, heating at 60 to 80°C causes germination activation of the spores (25).

Salt is of primary importance in cured meat products, and high concentrations of salt can exert an inhibitory effect on any microorganisms present in meat. The growth of *C. perfringens* at 30 to 37°C is not inhibited in broth media containing 4% NaCl, while some strains do not grow in the presence of 5 to 6% NaCl and most strains fail to grow in the presence of 7 to 8% NaCl (3). Juneja and Marmer (9) investigated the effect of NaCl concentration on the growth of *C. perfringens* from spore inocula in ground turkey containing 0.3% sodium pyrophosphate. The addition of 3% NaCl delayed growth for 12 h at 28°C. Growth was completely inhibited after 7 days at 15°C in the presence of 3% NaCl, while growth occurred at a relatively slow rate in the presence of 1 to 2% NaCl.

Since raw protein foods may be contaminated with *C. perfringens*, inadequate cooking or improper cooling may result in a potentially unsafe product. These factors have been the most frequently cited contributing factors in *C. perfringens* outbreaks (1, 2). As consumer demand for minimally processed refrigerated foods increases, cook-chill foods may pose a special public health concern as vehicles for *C. perfringens* infection (16). Since potential hazards are associated with the cooling of cooked foods, the U.S.

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† Mention of a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

‡ Portions of this research were presented at the Annual Meeting of Society for Industrial Microbiology in Philadelphia, Pennsylvania, 11 to 15 August 2002.

TABLE 1. Properties of ham used in cooling studies

Parameter	Value of parameter for ham sample		
	A	B	C
Moisture (%)	71.21	74.80	74.67
Protein (%)	17.06	16.28	17.43
Salt (%)	2.38	2.75	1.96
Sugar (%)	1.45	1.31	0.87
Sodium phosphate (%) ^a	0.21	0.29	0.20
Sodium erythorbate (ppm)	235	418	178
Sodium nitrite (ppm)	26	12	12
Water activity	0.961	0.955	0.968

^a As added sodium triphosphosphate.

Department of Agriculture (USDA) requires that the relative growth of *C. perfringens* not exceed 1.0 log₁₀ CFU/g during the cooling of certain meat and poultry products (24). Contamination of food with *C. perfringens* to a level of >10⁶ cells per g is thought to be sufficient to cause gastrointestinal illness (14).

Growth characteristics of *C. perfringens* in broth inoculated with vegetative cells (5, 10) and during growth from spore inocula in cooked, cured beef (11) and cooked, cured chicken (8) have been investigated. Relatively little information on the behavior of *C. perfringens* during the cooling of cooked meat products is available. The effects of the cooling rate on the outgrowth of *C. perfringens* spores in cooked ground beef (12), in cooked, ready-to-eat turkey breast roasts (19), and in cooked bologna (70% chicken), chunked ham with emulsion, and whole-muscle ham (22) have been investigated. Buffered sodium citrate at ≥1.0% was effective in reducing *C. perfringens* populations in roast beef and pork when cooling times after heat processing were extended to 21 h (23).

Information on the effects of various substances commonly incorporated into meat products as ingredients, curing agents, or antimicrobial agents on the fate of *C. perfringens* during the cooling of heat-processed meat products is needed. The effects of NaCl on the fate of *C. perfringens* during cooling of heat-processed ham and beef have not been reported. The objective of this research was to determine the influence of NaCl concentration and cooling rate on the outgrowth of *C. perfringens* spores in cooked ham and cooked ground beef.

MATERIALS AND METHODS

Microorganisms. Three strains of *C. perfringens* (NCTC 8238 [Hobbs serotype 2], NCTC 8239 [Hobbs serotype 3], and NCTC 10240 [Hobbs serotype 13]) from our in-house culture collection at the Eastern Regional Research Center were used in this study. Spore suspensions were prepared as described previously (4). A spore cocktail containing all three strains was prepared by mixing equal numbers of spores from each suspension.

Preparation of test materials. The test materials were smoked, cooked, cured boneless hams and raw ground beef. Hams A and B, cured with water, salt, sugar, sodium phosphates, sodium erythorbate, and sodium nitrite, were obtained from a commercial manufacturer. Ham C was prepared for this study by a commercial

TABLE 2. Cooling times and temperatures for cooked ground ham and cooked ground beef

Elapsed time (h)	Temp (°C) for cooling period		
	15 h	18 h	21 h
0	54.4	54.4	54.4
1	46.4	47.6	48.5
2	39.6	41.7	43.3
3	33.9	36.6	38.7
4	29.1	32.2	34.6
5	25.0	28.3	31.0
6	21.6	25.0	
7	18.7	22.1	25.0
8	16.2	19.6	
9	14.2		20.3
10	12.5	15.6	
11			16.6
12	9.8	13.9	
13			13.7
13.5	8.8		
14		12.5	
15	7.9		11.4
16		10.2	
17			9.7
18		8.5	
19			8.3
20			
21			7.2

manufacturer with the use of minimal curing ingredients (water, salt, sodium phosphates, sodium erythorbate, and sodium nitrite). The compositions of the hams, ground twice prior to use, are shown in Table 1. The analyses were performed by a commercial analytical laboratory with the use of standard methods. The raw ground beef (75% lean) was obtained from a local supermarket.

Ham A was supplemented with 0, 0.7, 1.2, and 1.7% NaCl to obtain levels of 2.4, 3.1, 3.6, and 4.1% NaCl, respectively. Ham B was supplemented with 0, 0.5, 1.0, and 1.5% NaCl to obtain levels of 2.8, 3.3, 3.8, and 4.3% NaCl, respectively. Ham C was supplemented with 0, 0.5, 1.0, and 1.5% NaCl to obtain levels of 2.0, 2.5, 3.0, and 3.5% NaCl, respectively. Sufficient NaCl was added to ground beef to result in levels of 0, 1, 2, 3, and 4%. Each batch of meat (500 g) was inoculated with the spore cocktail (1 ml) to a level of ca. 3 log₁₀ CFU/g and blended in a mixer (KitchenAid, Troy, Ohio). Portions of the inoculated meats (5 g each) were weighed into plastic bags (11 by 19 cm; BagPage Plus, Interscience, St. Nom, France). The contents of the bags were spread into a thin layer (ca. 1 to 2 mm) by pressing against a flat surface, excluding most of the air. The bags were then heat sealed under vacuum and stored frozen at -40°C for up to 6 months until they were used. To determine the initial spore densities for the test samples, randomly selected bags were heat shocked at 75°C for 20 min, and *C. perfringens* was enumerated as described below.

Cooling studies. Samples were thawed at room temperature and heat shocked at 75°C for 20 min. One set of samples (one bag for each NaCl level) was used for the determination of the initial population (0 h) of *C. perfringens*. Another set of samples was placed in a programmable water bath (Exacal, Model RTE-221, NESLAB Instruments, Inc., Newington, N.H.), equilibrated for 10 min at 54.4°C, and cooled from 54.4 to ≤8.5°C in 15, 18, or 21 h with the appropriate program (Table 2). The cooling pro-

TABLE 3. Effects of NaCl concentration and cooling rate^a on growth of *Clostridium perfringens* from spore inocula in cooked ground ham

NaCl concn (%)	Data for cooling period ^b							
	0 h		15 h		18 h		21 h	
	<i>n</i>	Mean <i>C. perfringens</i> count (SD)	<i>n</i>	Mean <i>C. perfringens</i> count (SD)	<i>n</i>	Mean <i>C. perfringens</i> count (SD)	<i>n</i>	Mean <i>C. perfringens</i> count (SD)
Ham A								
2.4	6	3.63 (0.22) B	2	4.42 (0.01) AB	2	4.64 (0.26) A	2	5.01 (0.40) A
3.1	6	3.66 (0.13) A	2	3.36 (0.21) A	2	3.54 (0.05) A	2	3.88 (1.01) A
3.6	6	3.63 (0.13) A	2	3.02 (0.33) A	2	3.07 (0.01) A	2	3.01 (0.27) A
4.1	6	3.56 (0.16) A	2	2.75 (0.48) AB	2	2.91 (0.11) AB	2	2.70 (0.00) B
Ham B								
2.8	3	3.53 (0.07) D	3	4.37 (0.04) C	3	5.48 (0.07) A	3	4.63 (0.04) B
3.3	3	3.46 (0.11) A	3	3.54 (0.03) A	3	3.57 (0.01) A	3	3.18 (0.02) B
3.8	3	3.39 (0.10) A	3	3.24 (0.02) AB	3	3.05 (0.06) B	3	2.66 (0.02) C
4.3	3	3.32 (0.11) A	3	3.12 (0.06) B	3	2.82 (0.04) C	3	2.61 (0.01) D
Ham C								
2.0	6	3.60 (0.02) B	8	4.00 (0.84) B	8	4.62 (1.18) AB	6	5.68 (1.17) A
2.5	4	3.49 (0.04) A	4	2.78 (0.07) B	4	2.38 (0.17) C	4	2.34 (0.10) C
3.0	4	3.42 (0.01) A	4	2.52 (0.04) B	4	2.15 (0.11) C	4	2.15 (0.00) C
3.5	4	3.35 (0.02) A	4	2.39 (0.04) B	4	2.05 (0.12) C	4	2.04 (0.16) C

^a Time for temperature to decrease from 54.4 to $\leq 8.5^{\circ}\text{C}$.

^b 0 h value represents initial population of *C. perfringens*. *n*, number of replicates. *C. perfringens* counts are given in log₁₀ CFU/g. Means with different letters in the same row are significantly different ($P < 0.05$; Bonferroni least significant difference test).

grams were derived from equations developed to simulate the exponential cooling of cooked foods (12).

Determination of *C. perfringens* populations. *C. perfringens* populations were determined immediately after heat shocking (initial populations) and after cooling for 15, 18, and 21 h. Bags were cut open, 5 ml of sterile 0.1% peptone water was added to each bag to produce a 1:1 (wt/vol) slurry, and the contents of the bag were homogenized for 2.5 min in a MimiMix (Interscience, St. Nom, France). Dilutions of the slurry were prepared with sterile 0.1% peptone water and surface plated onto tryptose-sulfite-cycloserine (TSC) agar without egg yolk enrichment (6) with a Spiral plater (Model D, Spiral Biotech, Bethesda, Md.). The agar plates were overlaid with additional TSC agar (5 ml) and incubated at 37°C for 48 h in an anaerobic chamber (Bactron, Sheldon Laboratories, Cornelius, Oreg.), and colonies were counted. Also, 0.1 ml of the undiluted slurry was spread over the surface of TSC agar. The lower limit of detection for this enumeration procedure was 20 CFU/g.

Statistical analyses. The cooling data and colony counts were subjected to analysis of variance to determine the effects of NaCl concentration and cooling rate on the population (log₁₀ CFU/g) of *C. perfringens*. Mean separations were carried out with the Bonferroni least significant difference technique.

RESULTS AND DISCUSSION

A survey of 10 hams available at a local supermarket revealed considerable variation in salt contents ($2.8\% \pm 0.8\%$ NaCl, with a range of 1.8 to 5.0% NaCl, as calculated from the product label values [milligrams of Na per serving]). Accordingly, cooling studies were conducted to assess the fate of *C. perfringens* from a spore inoculum in

two commercially prepared hams (hams A and B) and a specially formulated ham obtained from the same manufacturer that was prepared with minimal cure ingredients and met the USDA Food Safety and Inspection Service definition of cured ham (ham C) (Table 1). Cooling studies were also conducted with ground beef purchased at a local supermarket. These products were supplemented with NaCl at levels consistent with levels found in commercial cured meat products. These studies were designed to simulate the cooling process in the interior of ham or roast beef after the heat-processing step. For this purpose, thin layers of the products were heat shocked at 75°C for 20 min and then subjected to the cooling regimens (Table 2) to bring the temperature from 54.4 to $\leq 8.5^{\circ}\text{C}$. Heat treatment of the inoculated meat samples at 75°C for 20 min served to simulate the cooking of meat products and to heat activate the *C. perfringens* spores (23).

The initial populations of *C. perfringens* (0-h counts) were not significantly ($P < 0.05$) affected by the NaCl levels in the ham or in the beef; however, a significant NaCl level-cooling rate interaction was noted for the products studied (Tables 3 and 4). Results for cooked ground ham A are summarized in Table 3. *C. perfringens* counts for ham containing the lowest level of NaCl (2.4%) increased by 0.8, 1.0, and 1.4 log₁₀ CFU/g with cooling times of 15, 18, and 21 h, respectively. However, the bacterial population after 15 h of cooling was not significantly different ($P < 0.05$) from the initial population. No increases in *C. perfringens* counts were noted for ham containing 3.1, 3.6, and 4.1% NaCl with any of the three cooling programs. In fact,

TABLE 4. Effects of NaCl concentration and cooling rate^a on growth of *Clostridium perfringens* from spore inocula in cooked ground beef

NaCl concn (%)	Data for cooling period ^b							
	0 h		15 h		18 h		21 h	
	<i>n</i>	Mean <i>C. perfringens</i> count (SD)	<i>n</i>	Mean <i>C. perfringens</i> count (SD)	<i>n</i>	Mean <i>C. perfringens</i> count (SD)	<i>n</i>	Mean <i>C. perfringens</i> count (SD)
0	5	2.93 (0.31) c	3	7.20 (0.01) B	4	8.18 (0.11) A	3	8.33 (0.15) A
1	5	2.92 (0.23) c	3	5.84 (0.00) B	3	7.84 (0.11) A	3	8.12 (0.18) A
2	5	2.81 (0.19) B	3	3.51 (0.02) A	4	3.24 (0.20) AB	3	3.71 (0.38) A
3	5	2.81 (0.22) A	3	2.74 (0.07) A	4	2.36 (0.15) A	3	2.85 (0.24) A
4	4	2.66 (0.32) A	3	2.48 (0.09) A	3	2.15 (0.13) A	3	2.11 (0.44) A

^a Time for temperature to decrease from 54.4 to $\leq 8.5^{\circ}\text{C}$.

^b 0 h value represents initial population of *C. perfringens*. *n*, number of replicates. *C. perfringens* counts are given in \log_{10} CFU/g. Means with different letters in the same row are significantly different ($P < 0.05$); Bonferroni least significant difference test).

slight decreases in *C. perfringens* counts compared with the initial levels were observed for ham containing 3.6 and 4.1% NaCl. These decreases were not statistically significant except for ham containing 4.1% NaCl after 21 h of cooling.

Results for cooked ground ham B (Table 3) were generally similar to those for ham A. *C. perfringens* counts for ham containing the lowest level of NaCl (2.8%) increased by 0.8, 2.0, and 1.1 \log_{10} CFU/g with cooling times of 15, 18, and 21 h, respectively. Although for ham containing 2.8% NaCl, the level of *C. perfringens* observed after a cooling time of 15 h was significantly different ($P < 0.05$) from the initial level, the population increase of 0.8 \log_{10} CFU/g was still within the acceptable limit set by the USDA (24). Growth in ham containing higher concentrations of NaCl was not observed with any of the three cooling programs, and populations declined by up to 0.7 \log_{10} CFU/g with increasing NaCl levels and increasing cooling times.

Results for cooked ground ham C are shown in Table 3. Populations of *C. perfringens* in ham containing 2.0% NaCl increased by 0.4, 1.0, and 2.1 \log_{10} CFU/g with cooling times of 15, 18, and 21 h, respectively. The bacterial count after 15 h of cooling was not significantly different ($P < 0.05$) from the initial level; however, some of the replicates showed population increases of $>1 \log_{10}$ CFU/g after a cooling time of 15 h. In the presence of $\geq 2.5\%$ NaCl, growth was not observed and the bacterial populations were significantly smaller ($P < 0.05$) for all three cooling rates.

Results for cooked ground beef are shown in Table 4. Populations of *C. perfringens* increased with increasing cooling time for the lower levels of NaCl, reaching ca. 8 \log_{10} CFU/g in beef containing 0 and 1% NaCl during 18 and 21 h of cooling. *C. perfringens* counts increased to 7.2 and 5.8 \log_{10} CFU/g after 15 h of cooling in beef containing 0 and 1% NaCl, respectively. Minimal growth ($<1 \log_{10}$ CFU/g) was observed for all three cooling rates for beef containing 2% NaCl. No *C. perfringens* growth in beef containing 3 and 4% NaCl during cooling for 15, 18, or 21 h was observed.

Juneja et al. (12) studied the influence of cooling rate on the outgrowth of *C. perfringens* spores in cooked ground beef. The beef was cooked to an internal temperature of 60°C in 1 h, and then the samples were cooled from 54.4 to 7.2°C in 6 to 18 h. While the outgrowth of *C. perfringens* was not observed during the 6-, 9-, and 12-h cooling periods, minimal growth (ca. 1 \log_{10} CFU/g) was observed after the 15-h cooling period, and the growth level reached 6 \log_{10} CFU/g when the cooling period was extended to 18 h. These results differed from those obtained in the present study for beef without added salt, possibly because Juneja et al. (12) used a lower inoculum level (1.5 \log_{10} CFU/g) and a different strain in their inoculum cocktail. Beef products are the vehicles most frequently implicated in food poisoning due to *C. perfringens*, whereas cured meats are rarely implicated (18). From 1978 to 1992, only two outbreaks due to ham were reported to the U.S. Centers for Disease Control and Prevention (13). This low incidence of food poisoning caused by *C. perfringens* in cured meats suggests that the organism may grow poorly in the presence of salt and/or nitrite. All 21 strains examined by Roberts and Derrick (17) grew at 35°C in the presence of 4% NaCl in a pH 6.0 broth medium; however, lower levels of NaCl were found to inhibit *C. perfringens* when combined with NaNO_2 . *C. perfringens* did not grow from spore inocula in bologna, chunked ham with emulsion, or whole-muscle ham containing 2.4, 2.2, and 3.1% NaCl, respectively, even after extended periods of cooling from 54.4 to 7.2°C (22); however, these products contained relatively high levels of NaNO_2 (57 to 78 ppm, determined at peak cook temperatures) that might have contributed to growth inhibition. Vegetative cells of *C. perfringens* failed to grow on sliced chopped ham held at 30 or 21°C for 24 h (21), and variable results were obtained when vegetative cells of *C. perfringens* were inoculated onto freshly sliced ham and into chopped ham and stored at 30°C for 24 h (20).

Cooling times longer than those normally employed in the commercial preparation of ham were used in this study to allow us to examine the effect of a cooling process failure on the ability of *C. perfringens* spores to germinate and grow. Our data indicate that NaCl concentration has a pro-

found effect on the ability of *C. perfringens* to grow during the cooling of cooked meat products. Results obtained in the present study suggest that the cooling of cooked ham, which is normally formulated to contain >2% NaCl, to a temperature of <8.5°C in 15 h would yield an acceptable product. However, the cooling cooked beef under the same conditions may lead to an increase in *C. perfringens* levels sufficient to cause illness, particularly if the NaCl content of the beef is <2%.

ACKNOWLEDGMENTS

I thank Anna Kim-Rzepnicki for skilled technical assistance in conducting the bacterial survival studies and John G. Phillips for performing the statistical analyses. I also thank Vijay K. Juneja and Mark L. Tamplin for suggesting and facilitating this research project.

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